

## Recombinase-Mediated Reprogramming and Dystrophin Gene Addition in mdx Mouse Induced Pluripotent Stem Cells.

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**Authors:** Chunli Zhao, Alfonso P Farruggio, Christopher R R Bjornson, Christopher L Chavez, Jonathan M Geisinger, Tawny L Neal, Marisa Karow, Michele P Calos

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### Public Summary:

This paper demonstrates a complete strategy for a stem cell therapy for muscular dystrophy. In this study, we modeled our strategy in mice. We used cells from mice that have a mutation in the same gene, dystrophin, that is mutated in patients that have Duchenne muscular dystrophy. We started with skin cells from the mouse and used a new method to turn the skin cells into stem cells. This procedure, called "reprogramming", was done without using any viruses, and we did not place genes into the chromosomes at random. Instead, we placed the genes needed for reprogramming into a single, safe location in the mouse chromosomes. This was done by positioning the genes in the chromosome with a recombinase enzyme that recognizes specific DNA sequences. We used the recombinase in combination with a circle of DNA called a plasmid that carried the reprogramming genes. We verified the position by DNA sequencing and made sure that no genes were disrupted during the reprogramming process. Then we added a correct copy of the coding sequence for mouse dystrophin. To do this, we used a second recombinase enzyme that recognizes a specific DNA sequence that was present on the original reprogramming plasmid. We verified that the dystrophin gene was now present in the cells. The corrected cells were then turned into muscle precursor cells by growing the cells in a specific way in culture dishes. After about two weeks in cell culture, the cell population was sorted on an instrument that allowed us to purify cells that were making a protein typical of muscle precursor cells. We took the positive cells and injected them into a leg muscle in the disease model mice. We showed that these cells became incorporated into the muscle and expressed the dystrophin gene. Thus, this study provides a model for our strategy for a muscular dystrophy therapy. We are now doing similar experiments in human cells, starting with skin cells from muscular dystrophy patients. By using a strategy similar to the one we used in this mouse study, we hope to develop a therapy for human muscular dystrophies.

### Scientific Abstract:

A cell therapy strategy utilizing genetically-corrected induced pluripotent stem cells (iPSC) may be an attractive approach for genetic disorders such as muscular dystrophies. Methods for genetic engineering of iPSC that emphasize precision and minimize random integration would be beneficial. We demonstrate here an approach in the mdx mouse model of Duchenne muscular dystrophy that focuses on the use of site-specific recombinases to achieve genetic engineering. We employed non-viral, plasmid-mediated methods to reprogram mdx fibroblasts, using phiC31 integrase to insert a single copy of the reprogramming genes at a safe location in the genome. We next used Bxb1 integrase to add the therapeutic full-length dystrophin cDNA to the iPSC in a site-specific manner. Unwanted DNA sequences, including the reprogramming genes, were then precisely deleted with Cre resolvase. Pluripotency of the iPSC was analyzed before and after gene addition, and ability of the genetically corrected iPSC to differentiate into myogenic precursors was evaluated by morphology, immunohistochemistry, qRT-PCR, FACS analysis, and intramuscular engraftment. These data demonstrate a non-viral, reprogramming-plus-gene addition genetic engineering strategy utilizing site-specific recombinases that can be applied easily to mouse cells. This work introduces a significant level of precision in the genetic engineering of iPSC that can be built upon in future studies.